Research Note: Dietary resveratrol supplementation improves the hepatic antioxidant capacity and attenuates lipopolysaccharide-induced inflammation in yellow-feathered broilers

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ABSTRACT This experiment investigated the protective effect of resveratrol (**RES**) on the hepatic antioxidant status and systemic inflammation in yellow-feathered broilers challenged with lipopolysaccharide (**LPS**). A total of 240 healthy 1-day-old yellow-feathered broilers were randomly divided into 4 groups (control, LPS, RES, and RES+LPS), with 5 replicates of 12 chickens per replicate. The experiment lasted 21 d. The broilers were fed with either the basal diet or the basal diet supplemented with 400 mg/kg RES followed by intraperitoneal challenge with LPS (1 mg/kg body weight) or the same amount of saline at d 16, 18, and 20. The results showed that dietary RES supplementation could improve the activities of total antioxidant capacity (**T-AOC**) and superoxide dismutase (**SOD**) in the liver of yellow-feathered broilers challenged with LPS ($P < 0.05$). Furthermore, LPS challenge increased the plasma interleukin-17 (**IL-17**) concentration, the hepatic interleukin-6 (**IL-6**) and interleukin-1β (**IL-1β**) concentrations, as well as the concentrations of tumor necrosis factor (**TNF-α**), IL-6, and IL-1β in the spleen ($P < 0.05$), and decreased the transforming growth factor-β (**TGF-β**) concentrations in the plasma, liver, and spleen ($P < 0.05$). However, dietary RES supplementation could reduce the increased TNF-α levels in the plasma, liver, and spleen induced by LPS, and increased TGF-β level in the liver and spleen ($P < 0.05$). Collectively, these results suggest that dietary RES supplementation could effectively improve the hepatic antioxidant capacity and attenuate LPS-induced inflammation in yellow-feathered broilers during the starter stage.

Key words: yellow-feathered broiler, resveratrol, lipopolysaccharide, antioxidant capacity, inflammation

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INTRODUCTION

Poultry are constantly susceptible to various pathogens and stressors during modern intensive poultry production, which may affect the performance and health of poultry. Lipopolysaccharide (**LPS**) is the cell wall component of Gram-negative bacteria, and could induce oxidative stress and inflammation in poultry as an important stressor. However, dietary nutritional intervention (such as antioxidants) represents an effective approach to alleviate the stress responses or inflammation in poultry (He et al., 2019; Meng et al., 2022).

Resveratrol (**RES**, trans-3, 4', 5-trihydroxystilbene) is a natural polyphenolic compound widely distributed in many plants, and displays strong antioxidant, antibacterial, anti-inflammatory properties. Previous studies have shown that dietary RES supplementation improved the growth performance of broilers (Wang et al., 2021), ducks (Yang et al., 2021), and weaning piglets (Chen et al., 2021). Moreover, dietary RES treatment could prevent the impaired meat quality induced by transport stress through the enhancement of muscle antioxidant capacity in broilers (Zhang et al., 2017), and attenuated the intestinal development and antioxidant function of broilers under heat stress (Wang et al., 2021). Our previous study also showed that dietary RES supplementation might enhance the growth performance and intestinal antioxidant capacity in LPS-challenged broilers through modulation of gut microbiota composition (He et al., 2022). However, it remained largely unknown whether dietary RES supplementation could alleviate the LPS-induced hepatic oxidative stress and systemic inflammation in yellow-feathered broilers especially at early growth stage (1−21 d).

Thus, this experiment was carried out to investigate the protective effects of dietary RES supplementation on hepatic antioxidant status and cytokine secretions in yellow-feathered broilers challenged with LPS.

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Table 1. The composition and nutrient levels of the basal diet.

Ingredients	%	Calculated nutrient levels	
Corn	39.07	Metabolic energy, MJ/kg	12.35
Wheat	25.00	Crude protein, %	21.50
Soybean meal (43%CP)	22.00	Ca, %	0.90
Peanut meal	3.00	Total phosphorus, %	0.60
Corn gluten meal (58%CP)	5.00	L-Lysine, %	1.38
Limestone	1.45	DL- Methionine, %	0.61
$CaHPO_4$	1.10	L-Threonine, %	0.82
L-Lysine sulfate	0.64		
DL-Methionine	0.32		
Sodium chloride	0.28		
L-Threonine	0.19		
Lard oil	1.45		
Vitamin-mineral premix[1]	0.50		
Total	100.00		

[1]The premix provided the following per kg of diet: VA (all-trans retinol), 6,000 IU; VD_3, 2,000 IU; VE (dl-α-tocopherol), 33 IU; VK_3, 2 mg; VB_1, 3 mg; VB_2, 5 mg; pantothenic acid, 800 mg; choline chloride 1,500 mg; nicotinic acid, 30 mg; pyridoxine, 3 mg; folic acid, 500 mg; biotin, 0.2 mg; VB_{12}, 1 mg; Fe, 100 mg; Cu, 8 mg; Mn, 100 mg; Zn, 100 mg; I, 0.42 mg; Se, 0.3 mg.

MATERIALS AND METHODS

Ethics Statement

This experimental protocol was approved by the Ethical Committee and conducted under the supervision of the Institutional Animal Care and Use Committee of Foshan University (Foshan, China).

Experimental Design and Diet

A total of 240 healthy 1-day-old male yellow-feathered broilers were randomly divided into 4 groups. Each group had 5 replicates with 12 chickens per replicate. The broilers in control group and LPS group were fed with basal diet (Table 1), while those in the RES group and RES + LPS group were fed with the basal diet supplemented with 400 mg/kg RES (purity >98%, Shaanxi Sciphar Natural Products Co., Ltd., Xi'an, China). The experiment lasted 21 days. At d 16, 18, and 20 of the experiment, the broilers in LPS group and RES + LPS group were injected intraperitoneally with LPS (1 mg/mL body weight), while the broilers in control group and RES group received the same amount of saline. The LPS (*Escherichia coli* O55:B5, Sigma-Aldrich, St. Louis, MO) was dissolved in sterile saline. All birds had ad libitum access to feed and water throughout the study period. All broilers were maintained on a 18 h light and 6 h dark cycle in a controlled environment. The broilers in each replicate were reared in a single cage (32.5 × 62 × 42 cm). Ambient temperature was maintained at 34°C during the first week of the experiment and then gradually decreased to 25°C by 21 d.

Sample Collections and Determinations

After weighing at the morning of d 21, one broiler close to the average weight in each replicate was selected for sample collections. The blood samples were collected from the wing vein and then centrifuged at 1,320 *g*, 4°C for 10 min to harvest the plasma samples. After blood collection, the spleen and liver samples at the consistent locations were collected and frozen immediately in liquid nitrogen followed by storage at −80°C until analysis. Another portion of liver samples was collected for determining the hepatic antioxidant capacity. The antioxidant parameters including glutathione peroxidase (**GSH-Px**), superoxide dismutase (**SOD**), catalase (**CAT**), total antioxidant capacity (**T-AOC**), and malondialdehyde (**MDA**) were determined following the instructions of the manufacturer (Nanjing Jiancheng Bioengineering Inc., Nanjing, China). The levels of cytokines including tumor necrosis factor-α (**TNF-α**), interleukin-6 (**IL-6**), interleukin-1β (**IL-1β**), interleukin-17 (**IL-17**), and transforming growth factor-β (**TGF-β**) in the plasma, liver, and spleen were determined according to the instructions of the commercial kits provided by Beijing Fangcheng Biotechnology Co., Ltd (Beijing, China).

Statistical Analysis

All data were analyzed by two-way ANOVA followed by Duncan's multiple comparisons in IBM SPSS statistics software (version 25.0, Chicago, IL). The main effects of LPS challenge and RES treatment as well as the LPS × RES interactions were investigated. Data were expressed as mean ± standard error of the mean (**SEM**). The significance was declared at $P < 0.05$ and trends at $P < 0.10$.

RESULTS AND DISCUSSION

Hepatic Antioxidant Capacity

As shown in Table 2, LPS challenge increased the hepatic MDA concentration ($P = 0.01$), but dietary supplementation with RES did not affect the hepatic MDA concentration in yellow-feathered broilers ($P > 0.05$). Moreover, there was a significant main effect of RES in the hepatic SOD and T-AOC activities in yellow-feathered broilers, with higher hepatic SOD and T-AOC activities in the RES + LPS group when compared to the LPS group ($P < 0.05$). There was no difference in GSH-Px and CAT activities among treatments or LPS × RES interaction in these hepatic antioxidant parameters ($P > 0.05$). The balance between oxidation and antioxidation is of critical importance for maintaining the redox homeostasis, thus influencing the broiler health. The liver is an important target organ for responses to oxidative stress. The present study showed that dietary treatment with RES at 400 mg/kg might alleviate the hepatic oxidative stress by increasing hepatic activities of T-AOC and SOD in yellow-feathered broilers under LPS challenge. Our results were consistent with previous studies demonstrating the improvements of hepatic antioxidant status by dietary RES supplementation in weaning piglets (Chen et al., 2021) and quails (Sahin et al., 2012). Indeed, increasing evidence has confirmed the protective role of RES

Table 2. Effect of dietary resveratrol supplementation on the hepatic antioxidant capacity and cytokine levels in the plasma, liver, and spleen in yellow-feathered broilers challenged with lipopolysaccharide.

Item	Group				SEM	*P* value		
	Control	RES	LPS	RES+LPS		LPS	RES	LPS × RES
Hepatic antioxidant capacity								
GSH-Px (U/mg prot)	97.36	106.71	85.04	99.78	7.77	0.12	0.06	0.64
SOD (U/mg prot)	16.34^{ab}	18.12^{a}	13.60^{b}	18.09^{a}	1.34	0.18	0.01*	0.19
CAT (U/mg prot)	0.55	0.61	0.53	0.60	0.05	0.66	0.06	0.88
T-AOC (mmoL/mg prot)	36.93^{ab}	42.50^{a}	29.02^{b}	39.97^{a}	4.05	0.097	0.02*	0.40
MDA (nmoL/mg prot)	0.39 ^{b}	0.38 ^{b}	0.45^{a}	0.42^{ab}	0.02	0.01*	0.21	0.46
Plasma cytokines (pg/mL)								
TNF-α	1.27^{ab}	0.86^{b}	1.71^{a}	0.96^{b}	0.25	0.15	0.005*	0.36
IL-6	1.01	0.83	1.59	0.93	0.38	0.23	0.14	0.39
IL-1β	1.06^{ab}	0.70^{b}	2.46^{a}	0.92^{b}	0.68	0.06	0.02*	0.36
IL-17	1.92^{b}	1.29^{b}	3.95^{a}	2.38^{b}	0.54	0.003*	0.02*	0.25
TGF-β	6.23^{a}	7.47^{a}	4.23^{b}	5.73^{ab}	0.90	0.02*	0.06	0.84
Liver cytokines (pg/mL)								
TNF-α	366.87^{ab}	344.14^{b}	407.80^{a}	362.72^{ab}	21.10	0.06	0.04*	0.46
IL-6	270.39^{ab}	254.22^{b}	286.50^{a}	268.87^{ab}	8.78	0.03*	0.02*	0.91
IL-1β	168.60^{ab}	137.01^{b}	214.03^{a}	185.15^{a}	0.45	0.01*	0.06	0.92
IL-17	465.75	428.59	592.27	498.12	0.54	0.09	0.23	0.59
TGF-β	12.37^{b}	15.65^{a}	10.79^{b}	12.23^{b}	1.15	0.015*	0.02*	0.29
Spleen cytokines(pg/mL)								
TNF-α	3.52^{bc}	2.35^{c}	6.02^{a}	4.45^{b}	0.82	0.004*	0.046*	0.75
IL-6	9.73^{ab}	7.95^{b}	13.90^{a}	10.82^{ab}	2.08	0.04*	0.14	0.67
IL-1β	2.32^{ab}	1.57^{b}	3.96^{a}	2.86^{ab}	0.82	0.04*	0.15	0.77
IL-17	10.86	7.39	13.57	11.32	4.67	0.34	0.41	0.86
TGF-β	4.59^{ab}	7.60^{a}	2.77^{b}	4.33^{ab}	1.398	0.03*	0.049*	0.049*

Abbreviations: CAT, catalase; GSH-Px, glutathione peroxidase; IL-6, interleukin-6; IL-1β, interleukin-1β; IL-17, interleukin-17; LPS, lipopolysaccharide; MDA, malondialdehyde; RES, resveratrol; SOD, superoxide dismutase; T-AOC, total antioxidant capacity; TNF-α, tumor necrosis factor-α; TGF-β, transforming growth factor-β.

^{abc}Means in the same row with different superscripts differ ($P < 0.05$).

*Indicates $P < 0.05$.

against oxidative stress in different tissues of animals under heat stress or transport stress (Sahin et al., 2012; Zhang et al., 2017; Wang et al., 2021). For example, Sahin et al. (2012) found that RES treatment enhanced the activities of antioxidant enzymes (SOD, GSH-Px, and CAT) and decreased the MDA level in the hepatocytes of quail under heat stress. Moreover, dietary RES supplementation protected the broilers against high ambient temperature-induced spleen dysplasia through modulating splenic redox status and apoptosis (Zhang et al., 2018). Furthermore, dietary RES supplementation could attenuate the adverse effects of transport stress on muscle antioxidant capacity by increasing the T-SOD and GSH-Px activities and reducing the MDA content in the muscle of broilers (Zhang et al., 2017). Similarly, dietary RES treatment has been shown to improve the intestinal antioxidant function in broilers under heat stress (Wang et al., 2021). In accordance with these results, the current study indicated that RES could enhance the hepatic antioxidant capacity, which might help alleviate the LPS-induced hepatic oxidative damages in yellow-feathered broilers.

Cytokine Secretions in the Plasma, Liver, and Spleen

As shown in Table 2, compared to the control group, the plasma IL-17 concentration was increased while plasma TGF-β concentration was decreased after LPS challenge ($P < 0.05$). There was significant main effect of RES treatment in plasma concentrations of TNF-α ($P = 0.005$), IL-1β ($P = 0.02$), and IL-17 ($P = 0.02$) in yellow-feathered broilers. Specially, the plasma TNF-α, IL-1β, and IL-17 concentrations in RES+LPS group were decreased when compared to those in the LPS group ($P < 0.05$). Moreover, LPS challenge increased the hepatic IL-6 ($P = 0.03$) and IL-1β ($P = 0.01$) concentrations, and decreased the hepatic TGF-β concentration ($P = 0.015$) in yellow-feathered broilers. On the other hand, dietary RES treatment decreased the hepatic TNF-α ($P = 0.04$) and IL-6 (P=0.02) concentrations, and increased the hepatic TGF-β ($P = 0.02$) concentration in yellow-feathered broilers. The spleen is recognized as the biggest peripheral immune organ for poultry (He et al., 2019). For the spleen cytokines, the LPS challenge increased TNF-α ($P = 0.004$), IL-6 ($P = 0.04$), and IL-1β ($P = 0.04$) levels and decreased the TGF-β ($P = 0.03$) level in yellow-feathered broilers. However, dietary RES supplementation reduced the increased TNF-α concentration in the spleen of yellow-feathered broilers caused by LPS ($P < 0.05$). Furthermore, there was no differences in plasma IL-6 concentration as well as the IL-17 concentrations in the liver and spleen ($P > 0.05$). However, there was a significant LPS × RES interaction in TGF-β level in the spleen of yellow-feathered broilers ($P < 0.05$). To our knowledge, there were few studies investigating the anti-inflammatory effects of RES in LPS-challenged yellow-feathered broilers at early stages when they are susceptible to various stressors. Oxidative stress is closely related to the inflammatory responses by releasing the proinflammatory cytokines

(such as TNF- α, IL-1β, and IL-6). We found that dietary RES supplementation could reduce the elevated concentrations of TNF-α in the plasma, liver and spleen of yellow-feathered broilers induced by LPS, which indicated the beneficial effect of RES treatment on alleviating the LPS-induced systemic inflammation in yellow-feathered broilers during the starter phase. Our results were consistent with previous study that dietary RES supplementation could reduce the inflammatory response of ducks by decreasing the IL-1β and IL-6 levels in plasma and liver caused by LPS (Yang et al., 2021). Similarly, dietary RES supplementation has been demonstrated to regulate the innate immune and reduce the heat stress-induced inflammatory responses in the spleen of yellow-feathered broilers (He et al., 2019) as well as white-feathered broilers (Meng et al., 2022). Moreover, RES treatment by jugular injection attenuated the LPS-evoked inflammatory responses in lambs by suppressing expression levels of inflammatory cytokines including IL-1β, IL-6, and TNF-α (Liang et al., 2019). Consistently, previous study in weaning piglets also showed that dietary RES supplementation upregulated the mRNA expression of anti-inflammatory cytokine (*IL-10*) in jejunum mucosa (Chen et al., 2021). These aforementioned studies have confirmed the effectiveness of RES treatment in inhibition of inflammation-induced biomarkers. Therefore, our results indicated that dietary RES supplementation might protect the yellow-feathered broilers from LPS-induced inflammation mainly by reducing the secretions of inflammatory cytokines in the plasma, liver, and spleen during the starter stage (1−21 d).

In summary, the results suggested that dietary RES supplementation improved the hepatic antioxidant capacity, and attenuated LPS-induced inflammation by modulating the cytokine secretions in yellow-feathered broilers challenged with LPS at the early stage. These findings may provide insights into the future application of RES as functional feed additive in broilers and offer a potential nutritional strategy for alleviating the stress responses in modern poultry production. However, further investigations are necessary to elucidate the underlying mechanism concerning the protective effect of RES against LPS-induced hepatic oxidative damages and systemic inflammation in yellow-feathered broilers.

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DISCLOSURES

The authors declare that they have no competing interests.

REFERENCES

Chen, X. L., Z. Y. Zeng, Z. P. Huang, D. W. Chen, J. He, H. Chen, B. Yu, J. Yu, J. Q. Luo, Y. H. Luo, and P. Zheng. 2021. Effects of dietary resveratrol supplementation on immunity, antioxidative capacity and intestinal barrier function in weaning piglets. Anim. Biotechnol. 32:240–245.

He, S. P., Q. F. Yu, Y. J. He, R. Z. Hu, S. T. Xia, and J. H. He. 2019. Dietary resveratrol supplementation inhibits heat stress-induced high-activated innate immunity and inflammatory response in spleen of yellow-feather broilers. Poult. Sci. 98:6378–6387.

He, Z. T., Y. J. Li, T. D. Xiong, X. Y. Nie, H. H. Zhang, and C. Zhu. 2022. Effect of dietary resveratrol supplementation on growth performance, antioxidant capacity, intestinal immunity and gut microbiota in yellow-feathered broilers challenged with lipopolysaccharide. Front. Microbiol. 13:977087.

Liang, Y. P., J. W. Zhou, K. X. Ji, H. Liu, A. Degen, M. J. Zhai, D. Jiao, J. L. Guo, Z. S. Zhao, and G. Yang. 2019. Protective effect of resveratrol improves systemic inflammation responses in LPS-injected lambs. Animals (Basel) 9:872.

Meng, T. T., J. Y. Deng, D. F. Xiao, M. A. Arowolo, C. M. Liu, L. Chen, W. Deng, S. P. He, and J. H. He. 2022. Protective effects and potential mechanisms of dietary resveratrol supplementation on the spleen of broilers under heat stress. Front. Nutr. 9:821272.

Sahin, K., C. Orhan, F. Akdemir, M. Tuzcu, C. Iben, and N. Sahin. 2012. Resveratrol protects quail hepatocytes against heat stress: modulation of the Nrf2 transcription factor and heat shock proteins. J. Anim. Physiol. Anim. Nutr (Berl). 96:66–74.

Wang, C., F. Zhao, Z. Li, X. Jin, X. Y. Chen, Z. Y. Geng, H. Hu, and C. Zhang. 2021. Effects of resveratrol on growth performance, intestinal development, and antioxidant status of broilers under heat stress. Animals (Basel) 11:1427.

Yang, H., Y. J. Wang, M. Liu, X. Liu, Y. H. Jiao, S. J. Jin, A. Shan, and X. J. Feng. 2021. Effects of dietary resveratrol supplementation on growth performance and anti-inflammatory ability in ducks (*Anas platyrhynchos*) through the Nrf2/HO-1 and TLR4/NF-kB signaling pathways. Animals (Basel) 11:3588.

Zhang, C., K. K. Chen, X. H. Zhao, and Z. Y. Geng. 2018. Protective effects of resveratrol against high ambient temperature-induced spleen dysplasia in broilers through modulating splenic redox status and apoptosis. J. Sci. Food. Agric. 98:5409–5417.

Zhang, C., L. Wang, X. H. Zhao, X. Y. Chen, L. Yang, and Z. Y. Geng. 2017. Dietary resveratrol supplementation prevents transport-stress-impaired meat quality of broilers through maintaining muscle energy metabolism and antioxidant status. Poult. Sci. 96:2219–2225.